THE LOSS OF DIFFERENTIATION POTENTIAL OF HUMAN MESENCHYMAL STEM CELLS CAN BE PREDICTED BY USE OF A SET OF SENESCENCE MARKERS

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INTRODUCTION: Human mesenchymal stem cells (MSCs) are multipotent cells and have emerged as a promising tool for clinical applications. More than 40 years ago, Hayflick discovered that *in vitro* cultured cells, after a given number of divisions, become senescent [1]. Senescence also concerns MSCs, which, due to limited sample size, may have to be expanded *in vitro* to large extent for clinical use. From tissue engineering perspective, the senescence of expanded MSCs has certain implications, such as quality control and setting correct point for differentiation start. Mainly because of patient variability, it is unclear to what extend cell expansion can proceed for each case before the MSCs start to lose their ability to differentiate and whether senescing MSCs lose this ability gradually or as a discrete event [2]. Our aim was to develop a tool based on a set of parameters which can predict a future loss of differentiation capacity in order to provide optimal therapy andprevent waste.

METHODS: We assessed the senescence of hMSCs by monitoring cell division rate, colony forming units, senescence-associated beta-galactosidase activity and expression levels of three senescence-associated marker genes (CDC2, TOP2A and p53) across passages. This data was correlated to differentiation to chondrogenic phenotype of hMSCs cultured in 3-dimensional biodegradable scaffolds. Chondrogenesis of the constructs was assessed by RT-PCR (Aggrecan, Collagen Type II), immunoblotting as well as histological and immunohistochemical stainings.

RESULTS: Analysis of the data showed that hMSCs undergo *in vitro* senescence (*Figure 1*) which reduces their ability to differentiate (*Figure 2*). Senescence can be assessed by several markers but not a single one of them is 'fool proof'. Therefore, it makes sense to combine two or more markers in a battery of tests in order to predict excessive loss of potential to undergo chondrogenesis.

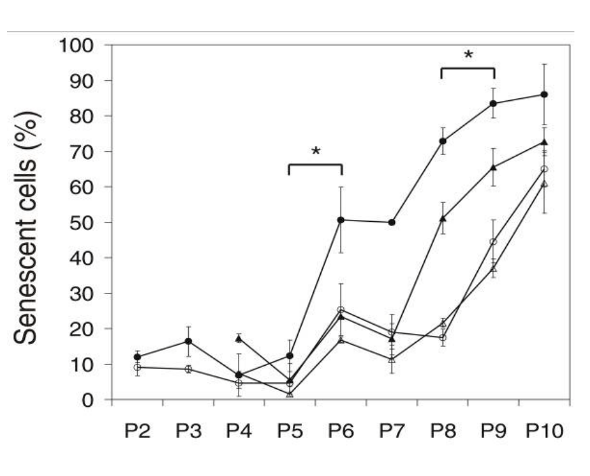

*Fig. 1: Percentage of senescent cells among passages. *, P<0.05 (paired Student's test).*

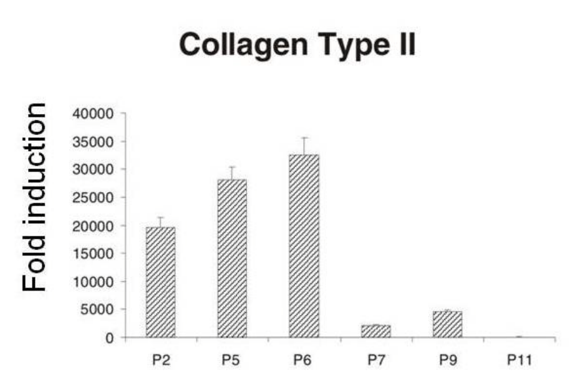

Fig. 2: Normalized expression of collagen type II after 30 days in chondrogenic medium, compared to control medium.

DISCUSSION & CONCLUSIONS: We demonstrated that it is possible to correlate senescence to differentiation at an earlier point when cells still can be used to their full potential. On practical level, it is worth cryopreserving consecutive passages and - once senescence evaluation accomplished - to use the appropriate ones for further differentiation.

REFERENCES: [1] Hayflick, Exp Cell Res, 1965; 37:614-36. [2] Roobrouck et al., Exp Cell Res 2008, 314:1937-44

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